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REVIEW ARTICLE

Microbial Xylanases: Genomics, Purification and Industrial Applications

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ABSTRACT
The hydrolysis of xylan, a kind of hemicellulose, is catalyzed by xylanase, which cleaves the molecules -1, 4 backbone and converts it to xylose, a simple sugar. Plant cell walls include xylan, which is one of the most significant components. Xylanases come in a variety of forms, each with its own mode of action, substrate specificities, and economic applications. Xylanases are important in industry because they can break down xylan into sustainable fuels and chemicals. Recent advances in recombinant protein engineering have enabled the construction and expression of xylanases in both heterologous and homologous hosts. Endo-xylanases have been successfully expressed in bacteria, yeasts, fungi, and plants, with yeasts proving to be the most promising. Food, paper & pulp, textile, and pharmaceutical sectors also use xylanases. The current review article explains how genomics may be utilized to enhance xylanase production, as well as how to clone and express xylanase genes into a suitable system for greater xylanase synthesis with desired characteristics, which can subsequently be used for a range of commercial applications.
Keywords: *Xylanase activity, Expression system, Cloning, Purification*

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INTRODUCTION

The principal components of plant cell walls are cellulose, hemicelluloses, and lignin, with cellulose being the most prevalent followed by hemicelluloses[1,2]. By dry weight, plant biomass contains 40 percent cellulose, 23 percent lignin, and 33 percent hemicellulose[3]. Biomass is a natural resource that may be used to make chemicals and feedstocks[4]. Annually, 830 Gt of sustainable plant biomass, mostly cellulose and hemicelluloses, is produced[5]. Enzymatic hydrolysis of biomass is gaining popularity ecofriendly. Various types of microbes that can degrade hemicellulose are isolated for the manufacture of xylanases[4]. Xylanase enzymes are purified, and genes encoding xylanases are cloned and described[6]. For xylanase production, a powerful microbe must be chosen, followed by medium optimization, purification, and enzyme characterization. Understanding the genetic components of bacteria will help us to figure out how enzymes work. With advances in technology and molecular biology, it is now feasible to isolate and clone the gene encoding xylanase in an appropriate environment, resulting in increased xylanase production with desirable features.

The rising need for xylanolytic enzymes in many industrial sectors necessitates a better understanding of xylan and xylanases. The majority of commercial xylanase operations require a thermostable and alkali stable enzyme. As a result, high thermostable and alkali stable xylanase with high activity is sought. In this study, a variety of molecular approaches have been explored to improve the physiological features of xylanases for wider commercial applicability in the industries in this genomic age.

GENOMICS OF XYLANASE

Xylanases were recognized in usual but efficient lignocellulose degradation microbiota, such as biogas digesters, the termite hindgut, the cow rumen, and the camel gut with the development of microbiome

profiling and other high-throughput sequencing technologies [7]. The termite hindgut is a proficient microbial niche for lignocellulose degradation, from where specific xylanases have been identified[7]. A whole of 913 microbial genomes and 69,000 genes related to carbohydrate metabolism were well-known through the assembly of cow rumen metagenomic sequences, characterizing the cow rumen as a reservoir of xylanase genes [8]. At least 386 various xylanase genes were screened from a single biogas digester, with xylanase activities detected by the expression of the recovered xylanase genes in *Escherichia coli* [9]. The artificial rumen system is greatly proficient in degrading lignocellulose for methane production[10], harboring various functional xylanases[11]. Moreover, many competent xylanase genes have been isolated from kangaroo and camel gut microbiota; some of them showed alkali-thermostable characteristics, which makes them useful in the augmentation of recalcitrant lignocellulose biomass degradation[12,13]. Paper and pulp manufacture wastewater contains elevated levels of lignocellulose waste, and the microbiota used to treat such wastewater harbors a diversity of lignocellulose genes. In the study reported by Wang et.al, over 93,000 genes predicted to be related with carbohydrate metabolism were known in the anaerobic and aerobic microbiota used for pulp and paper wastewater treatment (PPWT)[14].

CLONING AND EXPRESSION OF XYLANASE

An ideal xylanase should have specific properties to meet industrial needs such as stability over a wide range of pH values and temperatures, high specific activity, and strong resistance to metal cations and chemicals[15]. Cost effectivity, eco friendliness and ease of use are some other specifications of an ideal xylanase[16]. Native enzymes are not enough to meet all these demands due to small yields and incompatibility of the standard industrial fermentation processes. Consequently, molecular approaches are best way to design xylanases with all the necessary characteristics[17]. Heterologous expression is the major tool for manufacture of xylanases at industrial level[18]. Protein engineering by recombinant DNA technology could be useful in improving the specific characteristics of existing xylanases[17]. Large-scale expression of xylanases in homologous or heterologous protein is allowed by engineering and recombinant DNA technology in expression hosts. For industrial applications cheaper enzymes are required; elevation of expression levels and efficient secretion of xylanases is important for ensuring the viability of the process[19].

Expression in bacteria

Escherichia coli is known for its ease of manipulation, inexpensive growth conditions, simple techniques are required for transformation and accumulation of high levels of products in cell cytoplasm; therefore, this organism has become the most widely used expression host[20]. The expression of xylanase gene is done from *Bacillus brevis* in *E. coli* BL21[21]. The recombinant strain predominantly secreted xylanase in the culture medium with 30 IU/mL xylanase activity and the culture filtrate was free from cellulase activity and active in wide range of pH and temperature. Despite *Escherichia coli's* use as a good cloning host for recombinant proteins, it does not provide efficient and functional expression of many xylanases[22,19], and not all genes are easily expressed in *E. coli*. The problem may be due to the repetitive appearance of rare codons and the requirement for specific translational modifications, such as disulphide bond formation and glycosylation[19]. Therefore, this microorganism is not very useful for the detailed study of xylanase gene structure and for the improvement of the enzymes via protein engineering[18].

Lactobacillus species and *Bacillus* species have been attractive hosts for production of heterologous proteins, obtaining higher expression levels than *E. coli.*[23,20]. *B. subtilis* and *Lactobacillus* are Gram positive and perform N-glycosylation[24]. Their primary interest in industry and research is due to the fact that they are non-toxic and are generally recognized as safe (GRAS) [23, 19]. Members of the genus *Bacillus* (Gram positive bacteria), unlike *E. coli* (Gram negative bacteria) do not contain endotoxins (lipopolysaccharides), which are difficult to remove from many proteins during the purification process. The secretory production could also be advantageous in industrial production[25].

Expression in fungi and yeast

Filamentous fungi are capable commercial producers of xylanases, via both heterologous and homologous gene expression, and reach high expression yields with their own promoters[26]. Fungal gene expression can result in high yield of recombinant gene products[27,28]. The application of fungi as an expressing agent is cheaper as compared to bacteria due to low-cost substrate and downstream processing. Xn2 xylanase gene was expressed in *Trichoderma ressei* by homologous expression resulting in the 1.61 g/L of xylanase 2 on glucose containing medium[29]. Heterologous proteins are expressed in Yeasts. Yeast systems provides further benefits over bacterial expression systems in expression of proteins such as ability to cultivate to very high cell densities, capability to carry out eukaryotic post-translational

modifications, and capability to secrete proteins into fermentation media. *Hansenula polymorpha*, *Saccharomyces cerevisiae* and *Pichia pastoris* are commonly used for heterologous protein expressions[30]. Favorite host for expressing recombinant proteins in yeast is *S. cerevisiae* (Baker's yeast) and it was first described in 1981. *S. cerevisiae* has various limitations that led to reduced applications as a host for heterologous expression. These are hyperglycosylation of secreted proteins, unsteadiness of recombinant plasmid DNA, fermentative mode of growth and retention of proteins within periplasmic space declining yields of protein expression[30]. But still a large number of xylanases from filamentous fungi *Aspergillus nidulans*, *A. kawachii, Trichoderma reesei* and *A. niger* were successfully expressed in this yeast. The AOXI promoter is repressed by both glycerol and glucose in *P. pastoris* whereas the MOX promoter is repressed by glucose in *H. polymorpha* and it is de repressed to about one-fourth of the induced levels by glycerol. Aerobic growth with methanol induction ends up in high levels of recombinant proteins with AOXI or MOX promoter systems [30,31,32]. Multi-copy strains with the recombinant gene under the AOXI or MOX promoter control could be the choice for industrial scale protein expression. The over-expression of endoxylanases in *P. pastoris*, one of the most effective hosts for expressing eukaryotic genes, was shown to have a negative impact on cell physiology activating stress response thereby lowering the expression level (Prof. Anton Glieder, personal communication). Oleaginous yeast *Yarrowia. lipolytica* is an upcoming protein expression host. Application of this organism for heterologous protein expression has multiple advantages such as ability of this organism to metabolize glucose, alcohols, acetate and hydrophobic substrates such as alkanes, fatty acids and oils, well-characterized secretory system that yields high levels of recombinant proteins (i.e. 2 g l−1), glycosylation resembling mammalian system, easy screening for multi-copy strains using defective selection marker and usage of a single integration site. Secretory expression of *Humicola insolens* xylanase I from XPR2 promoter ended at 2 mg l−1 of the protein[33,31]. *Kluyveromyces lactis* is known for its applications as a host for expressing heterologous proteins related to food and dairy industries, i.e., bovine chymosin. As a recombinant protein expression platform, it furnishes with several advantages such as easy genetic manipulation, application of both integrative and episomal expression vectors, availability as a commercial kit from New England Biolabs, simple fermentation equipment and availability of super secreting strains. Xylanases from *Neocallimastix frontalis*, *Dictyoglomus thermophilum, H. insolens* and *Thermotoga* species have been successfully expressed at up to 130 mg ml−1[34]. Expression and characterization of a thermostable xylanase gene xynA from thermophilic fungus *Thermomyces lanuginosus* SY2 in *Pichia pastoris* was demonstrated[35].. The recombinant strain showed highest xylanase activity of 113.5 IU/mL and the xylanase activity retained above 80% at wide range of pH (2.5 to 8.5) for 48hours. Cloning and expression of xylanase gene xynf11a of *Aspergillus fumigatus* MKU1 in *Pichia Pastoris* X33[36] resulted in recombinant strain showing high xylanase activity (14U/ml) after 96 hours of growth. The recombinant xylanase was free of cellulase activity and hence it could be potentially used for pretreatment of paper pulp before bleaching.

Xylanase Purification

An appropriate substrate selection and nutritive media composition is of great importance for the successful production of xylanases. The substrate not only serves as carbon and energy source, but also provides the necessary inducing compounds for the enzyme production[37]. Cost of enzyme production increases if purified xylan used as a substrate to induce xylanase synthesis. Therefore, attempts have been done to develop a bioprocess to produce xylanase for commercial applications in high quantities from simple and inexpensive substrates. Generally standard column chromatographic techniques have been used in xylanase purification schemes. Ion exchange and size exclusion are the generally utilized schemes for xylanase purification, but there are also reports of purification with hydrophobic interaction column chromatography. The low molecular weight of certain xylanases has also enabled their separation from other proteins using ultra filtration. Purification of two proteins from culture supernatant of *Bacillus circulans* AB 16 through ion exchange and gel filtration yielded 40% enzyme recovery while purification in other *Bacillus sp*. yielded percentage recovery of 14% and 28%[38]. For potential applications on pulp fibers, the purification of xylanases is considered to be incomplete as long as cellulase activity is totally eliminated. **Table 1.** shows various purification methods used for xylanase production.

Table 1. Purification methodologies employed for xylanase from different microorganisms

Microorganisms	Purification Methodology	Fold Purification	Reference
Bacillus sp. SV-34S	Ammonium sulphate precipitation (80%)	10.62	[39]
	Carboxymethyl Sephadex C-50 cation exchange chromatography	12.94	
Bacillus arsenicilenatis DSM 15340	Ammonium sulphate precipitation (35-80%)	1.37	[40]
	DEAE Sepharose FF	3.06	
Arthrobacter sp.	Ammonium sulphate	2.0	[41]
	Sephadex G-200	3.5	
	DEAE- Sepharose FF	5.5	
	CM- Sepharose FF	21	
Thermotoga thermarum	Ni-affinity chromatography	6.9	[42]
Penicillium glabrum	Ammonium sulphate fractionation	3.25	[43]
	Molecular exclusion chromatography (Sephadex G-75 column)	5.10	
Aspergillus tamariikita	Single step purification Carboxymethyl cellulose (CM-cellulose chromatography)	7.43	[44]
Aspergillus flavus	Ammonium sulfate fractionation (40-60%)	2.7	[45]
	Gel Filtration (Hi Prep 16/60 Sephacryl S-100 HR column) chromatography	3.7	
Bacillus sp. GRE7	Ammonium sulfate fractionation (40-80%)	3.9	[46]
	DEAE-Cellulose	11.9	
	Sephadex G-75	28.5	

Applications of Xylanase

Xylanase commercial applications have increased significantly worldwide, over the past few years[47,48,49]. Due to applications of microbial xylanases in various industrial processes such as food, feed and pulp and paper industry they have attracted a great deal of attention. Also, they have shown an immense potential for increasing the production of several useful products such as SCPs, enzymes, liquid or gaseous fuels and solvents and sugar syrups, which can be used as such or as feed stock for other microbiological processes in a most economical way[50]. Xylanases are now considered as "one of the most industrially important enzymes"[49]. Table 4 shows commercial applications of microbial xylanases in various industries.

Table 2. Commercial applications of microbial xylanases in various industries: -

Industry	Applications	Functions	Reference
Food and feed	Clearing of fruits and vegetable juices	Improves maceration and Juice clarification, reduces viscosity. Improved extraction yield and filtration, process performance and product quality	[51]
	Dough and bakery	Xylanases hydrolyses arabinoxylans present in wheat flour thereby improving elasticity and strength of the dough, allowing easier handling, larger loaf volumes and improved bread texture.	[52, 53]
	Animal feed	Aids in reducing the intestinal viscosity and improving the utilization of proteins and starch by reducing the content of non-starch polysaccharides, thereby improving animal performance, digestibility and nutritive value of poorly degradable feeds such as barley and wheat	[54,55]
Detergent	Enhance cleaning ability of detergents	It improves the cleaning ability of detergents that are more efficient in cleaning fruit, vegetable, soils and grass stains	[49]
Bio-refinery	Biofuel production	Ethanol and xylitol production from	[56]

		lignocellulosic biomass by delignification process	
Paper and pulp	Bio-bleaching of kraft pulps	Reduces chlorine consumption and toxic discharges in effluents	[57]
	Bio- mechanical pulping	Facilitates the pulping process and reduces the use of mechanical pulping methods and hence reduce energy consumption	[57]
	Bio- modification of fibers	Improves process efficiency by increasing fibrillation and drainage properties of pulp and the paper strength	[58]

CONCLUSION

The main goal will be to have a better understanding of the molecular characteristics of xylanase and to clone it into appropriate expression vectors. This is because novel commercial applications of xylanases have been discovered, necessitating the development of xylanases that are stable and active throughout a wide pH and temperature range. Bioprospecting for novel genes, rational engineering, and directed evolution of established genes are potent strategies that may be utilized to enhance these enzymes because natural enzymes do not meet all of the process requirements. Because xylanase is advantageous to us both economically and environmentally, new ways for producing xylanases will be developed in the near future to meet the demands of diverse businesses.

FUTURE PERSPECTIVE

Because of its enormous capacity to hydrolyze xylan, xylanase is in high demand in industry. These xylanases are required in large numbers in order to conduct research in the industries. Due to a lack of adequate circumstances for microbe growth or incompatible fermentation parameters, xylanases are generated in extremely tiny amounts. The difficulty of large-scale production at low cost still exists. Cloning is therefore employed to boost the output of the industrially important enzyme xylanase. For effective xylan hydrolysis, cellulase-free xylanases are important. This kind of xylanase is produced by *Bacillus pumilus, Aspergillus fumigates*, and *Bacillus firmus*. Improved xylanase usage would benefit from the optimal temperature, pH stability, and carbon supply. Furthermore, when the xylanase-producing gene is cloned using recombination technology, xylanase production may be enhanced, and the recombinant strain's qualities are better to the native or crude enzyme. The immobilization strategies would boost xylanase enzyme synthesis even further. The specific activity of xylanase preparations is significantly lower than that of commercial amylases, proteases, and glucose isomerases. The task of finding, or at the at least boosting, the strain capable of producing a high specific activity xylanase is critical, but it may not be easy due to the heterogeneous character of the substrate – xylan. The substrate is separated into soluble and insoluble physical states in addition to chemical heterogeneity, making catalysis an extraordinarily complicated event. The xylanases may have multiplied as a result of the catalytic complexity. So, the xylanase multiplicity must be examined while taking into account the substrate's microheterogeneity; making it feasible to create a xylanase combination with higher specific activity than the separate components.

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